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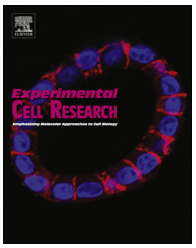

Research Article

MicroRNA-29b regulates TGF-β1-mediated epithelial–mesenchymal transition of retinal pigment epithelial cells by targeting AKT2

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ABSTRACT

The role of microRNA (miRNA) in proliferative vitreoretinopathy (PVR) progression has not been studied extensively, especially in retinal pigment epithelial–mesenchymal transition (EMT) which is the main reason for formation of PVR. In this study, we first investigated the miRNA expression profile in transforming growth factor beta 1 (TGF-β1) mediated EMT of ARPE-19 cells. Among the five changed miRNAs, miR-29b showed the most significant downregulation. Enhanced expression of miR-29b could reverse TGF-β1 induced EMT through targeting Akt2. Akt2 downregulation could inhibit TGF-β1-induced EMT. Furthermore, inhibition of miR-29b in ARPE-19 cells directly triggered EMT process, which characterized by the phenotypic transition and the upregulation of α-smooth muscle actin (α-SMA) and downregulation of E-cadherin and zona occludin-1 (ZO-1) with increased cell migration. Akt2-shRNA also inhibited miR-29 inhibitor-induced EMT process. These data indicate that miR-29b plays an important role in TGF-β1-mediated EMT in ARPE-19 cells by targeting Akt2.

Introduction

Epithelial–mesenchymal transition (EMT), a process of converting epithelial cells to mesenchymal cells, plays a crucial role in many physiological processes, such as the differentiation and repair of multiple tissues and organs [1–5]. At present, an increasing number of studies are demonstrating that EMT also contributes to various pathological processes, such as organ fibrosis [2,3], tumorigenesis and metastasis [4–6]. Proliferative vitreoretinopathy (PVR), characterized by the formation of preretinal and epiretinal membranes through the wound repair process, occurs in 5–10% of all rhegmatogenous retinal detachment (RRD) including untreated giant rhegmatogenous retinal detachment and long-standing retinal detachment. It is also implicated in redetachment after surgery in 70% of cases [7]. Recurrent preretinal or epiretinal membrane traction can further lead to retinal redetachment and significant visual loss [8–10]. Until now, there has been no satisfactory treatment for PVR. Though the mechanisms of PVR development are not completely understood, the conversion of retinal pigment epithelial (RPE) cells to mesenchymal cells

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through the EMT process is considered to be the main pathophysiological mechanism. A number of studies have observed the prevention of PVR by inhibiting EMT [11,12]. Our previous study verified that TGF-β1 could induce EMT in human RPE cells and that transcription factor Snail played a pivotal role in this process [11]. However, the molecular mechanism underlying TGF-β1-induced EMT is poorly understood.

MicroRNAs (miRNAs) are endogenous, non-coding single strand RNAs with approximately 22 nucleotides. They negatively regulate the expression of a wide variety of genes, mainly through direct interaction with the 3′-untranslated regions (3′UTR) of their corresponding mRNA targets [12]. Recent studies have demonstrated that miRNAs play an important role in both maintaining homeostasis and the development of disease [13–16]. In EMT and the organ fibrosis process, miR-200 family is reported to regulate TGF-β1-induced renal tubular EMT through the Smad pathway by targeting ZEB1 and ZEB2 [17]. MiR-29 family has also been shown to be highly related to the organ fibrosis. In renal fibrosis induced by Angiotensin II (Ang II), miR-29b was downregulated, while TGF-β, mesenchymal marker α-SMA and Collagen were upregulated. These changes promoted the renal fibrosis process [18]. Other study shows that miR-29 is a downstream inhibitor of TGF-β/Smad3 in TGF-β-mediated renal fibrosis [19]. But in fibrotic liver, miR-29 did not play an important role in the fibrosis regulated by Hedgehog signaling, when the NF-κB pathway is disrupted [20]. So we are more interested in the function of miR-29 in the fibrosis process.

In the retina, there exists more than 250 kinds of miRNAs which participate in retinal development, function, and disease [21]. For example, in retinal degeneration, expression of miR-96,–182 and -183 is shown to be decreased by 14.1–53.2%, while expression of miR-1, −133 and −142 is upregulated by 186.1–538.5% [22]. Another study suggests that miR-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy [23]. While the importance of miRNAs in retinal diseases is starting to be recognized, their roles in PVR remain largely unknown. Therefore, to elucidate the mechanisms behind PVR and enhance its diagnosis and treatment in a clinical setting, the role of miRNAs in its pathophysiology must be urgently understood.

In this study, we reported that miR-29b, a member of the miR-29 family, was downregulated in TGF-β1-induced EMT of ARPE-19 cells. MiR-29b played an important role in TGF-β1-induced EMT by targeting Akt2. Our results also suggested that downregulation of miR-29b may initiate the EMT process in ARPE-19 cells through Akt2.

Materials and methods

Cell culture

Human retinal pigment epithelial cells (ARPE-19) were obtained from the Eye Institute, Shanghai Jiao Tong University. 293T cells were kindly provided by Xiaoqing Liu's Laboratory at Tongji University. ARPE-19 cells were cultured in DMEM/F12 (Invitrogen Corporation, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen Corporation, Carlsbad, CA, USA). 293T cells were cultured in DMEM with high glucose in the presence of 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin (Solarbio, Shanghai, China). Cells were incubated at 37 °C in a humidified incubator containing 5% CO_2. The cell culture medium was changed every 3–4 days. For TGF-β1 treatment, ARPE-19 cells were seeded on 6-well plates at 80% confluence in cell culture medium with 10% fetal bovine serum. After 24 h, cells were subjected to serum-free medium and incubated for 16 h. TGF-β1 (Humanzyme, Chicago, IL, USA) was then added at a concentration of 10 ng/ml. Cells were maintained for 48 h and collected for the further analysis.

MicroRNA microarray

ARPE-19 cells treated with 10 ng/ml TGF-β1 for 48 h and the control cells were collected for miRNAs analysis with Agilent human miRNA (8 × 60K) V16.0. Microarray experiments were performed at Shanghai Biochip Company (Shanghai, China) according to the protocol of the Agilent miRNA microarray system. Briefly, total RNA was extracted with a mirVanaTM miRNA Isolation Kit (Ambion, Austin, TX, US) and labeled with a miRNA Complete Labeling and Hyb Kit (Agilent technologies, Santa Clara, CA, USA). After hybridizing with a miRNA Complete Labeling and Hyb Kit (Agilent Technologies), agilent Scan Control software was used for scanning the microarray slides. The experiment was performed in triplicate. The microarray data was analyzed using GeneSpring GX v11.0 software (Agilent Technologies).

RNA transfection

MiR-29b mimic, miR-29b inhibitor and their negative controls were purchased from Ribobio Company (Shanghai, China). For functional analysis, ARPE-19 cells were transfected with 100 nM miR-29b mimic and 200 nM miR-29b inhibitor using lipofectamine 2000 reagent (Invitrogen Corporation, Carlsbad, CA, USA) following the manufacturer instructions. Cells were incubated for 48 h after transfection and collected for experiments.

Plasmid DNA transfection

The plasmids of Akt2-targeting short hairpin RNA (shRNA) and control-shRNA were obtained from GeneChem Company (Shanghai, China). Plasmids and miR-29b inhibitor were cotransfected into ARPE-19 cells, at 80% confluency, using a HET kit (Biowit Technologies, Guangzhou, China) according to the manufacturer's protocol. Cells were incubated for 48 h after transfection.

Quantitative real-time RT-PCR (qRT-PCR)

The total RNA of cells was extracted using TRIzol reagent according to the manufacturer's instruction (Invitrogen Company, Carlsbad, CA, USA). The concentration of RNA was measured by Nano-drop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and cDNA was synthesized from 500 ng total RNA using a reverse transcription kit (Takara, Ohtsu, Shiga, Japan). Specific primers (GENTEC, Shanghai, China) were used to detect the EMT-related genes expression, with GAPDH used as an internal control. The bulge-loop microRNA primers (Ribobio Company, Guangzhou, China) were used to analyze the expression of microRNAs while the U6 primer (Ribobio Company, Guangzhou, China) served as an internal control. MiRNA quantification was performed using the 7500 Fast Real-time PCR System

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